A Novel Method for Synthesis of 2’-SeMeANA-dT

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A NOVEL METHOD FOR SYNTHESIS OF 2’-SeMeANA-dT

by

LINGRUI ZHENG

Under the Direction of Zhen Huang, PhD

ABSTRACT

Nucleic acids are fundamental building blocks of living systems’ genetic information, and are also responsible for regulating organisms’ protein synthesis, catalysis, etc. To better understand nucleic acids’ functions and working mechanisms, it is essential to obtain their structures. So far, the most common and powerful method to reveal the structures of macromolecules is X-ray crystallography. However, for nucleic acids structure determination, two problems are to be solved using such technology – crystallization and phasing. Selenium modification of nucleic acids has been proven to help address the two problems from native ones, without drastically affect their conformations. Also, previous studies suggest selenium modification enhances specific base pairing, especially against U-G wobble pair. With such feature, modified nucleic acids may be utilized by sequence-based diagnostics and therapies.

In this thesis, synthesis of 2’-arabino SeMe modified thymine is discussed. Such modification is believed to be compatible with B-form DNA structure determination.

INDEX WORDS: Nucleic acid, Thymine, Selenium, Synthesis, Mechanism, Dimethyl diselenide
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LINGRUI ZHENG

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Office of Graduate Studies
College of Arts and Sciences
Georgia State University
August 2018
DEDICATION

This thesis is dedicated to my parents, Hong Bu, Ruoping Li, and Jin Zheng. Thank you for raising me and providing the best environment for me to grow up. Your love has always been my strength when I’m down. You have guided me through my life and I appreciate the support, both emotional and physical.

I further dedicate this thesis to my grandfather, Ting Zheng. I would like you to know that your grandson has become, as Chinese people would say, a useful man to society. You are forever missed.
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Through my two years of graduate studies and research, I have experienced so much. This part of my life is definitely one of the most memorable times.

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1 INTRODUCTION

In 1958, Francis Crick stated the central dogma, which explains the flow of genetic information of a living system\textsuperscript{[1]}. Such transfers of biological sequential information rely on nucleic acids (NA), including DNA and RNA, which have been playing a significant role in the storage, transcription, and translation process in the central dogma, as well as protein regulation and catalysis, etc. While protein related research, diagnostics, treatment, and industry have been maturely developed, given NA’s role as a source and carrier in the flow of information, increasing attentions are drawn from biology, chemistry, and medical fields.

So far, X-ray crystallography is the most successful and influential tool in macromolecule structure determination. However, two problems, especially with NA, emerge and hinder the acquisition of the result, which are crystallization and phasing. As the name states, X-ray crystallography requires macromolecules to form crystals before their structures can be determined, but crystallization is exactly the speed-limiting step. Especially for native NA, it may take months or years to grow crystals, or it just may not give crystals, not to mention uniform and large-sized ones that are required for good diffraction data. To make things more complicated, tremendous amount of screening and trials are also needed to yield good crystals. Moreover, X-ray crystallography can only provide one of the two parameters in electron cloud density calculation, and therefore some measures must be carried out to obtain the unknown.

In 2001, Huang, Egli, et al. stated the concept of selenium modified nucleic acid (SeNA)\textsuperscript{[2,3,4]}, and with selenium’s elemental properties and so-called multiple wavelength anomalous diffraction (MAD phasing), the two problems from X-ray crystallography are addressed.
Both in Family VIA on the periodic table, selenium and oxygen share quite a lot of chemical and electron orbital properties. Thus, theoretically, substitution of oxygen in NA with selenium can be compatible for structural determination and properties enhancements, without changing native NA’s sugar pucker and/or stability drastically.

Figure 1.1 Selenium Modification Positions on NA

1.1 Purpose of the Study

As one of the modifiable positions, 2’-arabino modification of thymine is focused in this thesis. Previous studies have shown that 2’-alpha modification enhances crystallization capability of A-form DNA\textsuperscript{[5,6]}. Therefore, it is suspected that 2’-arabino position modifications may enhance that of B-form DNA, which is the most common DNA structure in living systems. However, 2’-SeMeANA-dG, although allowing modified DNA to be crystallized under broad crystallization conditions within significantly shorter period than the native, it also lowered the melting temperature of the modified DNA, a.k.a. destabilized it. Therefore, other NAs need to be tested to see if they work better. In this thesis, the preliminary step – synthesis of 2’-SeMeANA-dT is studied.
2 EXPERIMENT

2.1 Synthesis Using Previous Developed Scheme for 2'-SeMeANA-dU

![Chemical Diagram](image)

Figure 2.1 Initial Attempted Synthesis Scheme for 2'-SeMeANA-dT

2'-O-Trimethylsilyl-3',5'-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diy1)-thymine (2)

5-Methyluridine (1) 5g (19.4 mmol, MW: 258.23) was placed in a round bottom flask and dried under vacuum for 20 min, and applied argon balloon. It was then dissolved in 120 mL of pyridine and placed in ice bath. 4.4 mL of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (20.4 mmol, 1.05 eq, MW: 315.43, d: 1.454) was added dropwise while stirring. The flask was removed from ice bath and stirred for 3 hrs at room temperature. After reaction completed (determined by TLC), 7.37 mL (58.2 mmol, 3 eq, MW: 108.6, d: 0.856) of trimethylsilyl chloride was added dropwise and stirred overnight. The solution was evaporated to remove most of pyridine and then diluted by dichloromethane (DCM), and was then washed by 3M HCl, saturated sodium bicarbonate (NaHCO₃) solution, and saturated NaCl solution, respectively. It
was then dried with MgSO₄ and filtered and evaporated. After purified by silica gel column, the product was obtained as white foam.

3’,5’-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-N3-(tert-Butoxycarbonyl)-thymine (4) 5.5 g Compound (2) (9.6 mmol, MW: 572.9 g) was placed in a round bottom flask and dried under vacuum for 20 min. It was then dissolved in 90 mL of tetrahydrofuran (THF) and added 1.17 g (9.6 mmol, 1 eq, MW: 122.2) of 4-Dimethylaminopyridine (DMAP) and 2.65 mL (11.5 mmol, 1.2 eq, MW: 218.25, d: 0.95) of Di-tert-butyl dicarbonate (Boc). The solution was stirred 12 hrs under room temperature and after complete reaction (determined by TLC), it was added 3.65 g (19.2 mmol, 2 eq, MW: 190) of p-Toluenesulfonic acid monohydrate (p-TsOH) and stirred for 30 min. The solution was then cooled down to 0 ℃ and added 5.35 mL (38.4 mmol, 4 eq, MW: 101.19, d: 0.726) of triethylamine (TEA) dropwise and stirred for 10 min. The solution was filtered and washed with saturated NaHCO₃ solution and extracted with ethyl acetate (EA) three times. The organic layer was then collected and dried over anhydrous MgSO₄, filtered, and evaporated. After purified by silica gel column, the product was obtained as faint yellow/white solid.

2’-O-Mesy1-3’,5’-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-N3-(tert-Butoxycarbonyl)-thymine (5) 1 g (1.66 mmol. MW: 600.86) of Compound (4) was dried under vacuum for 20 min and applied argon balloon. It was then dissolved in 20 mL DCM and added 0.26 mL (3.32 mmol, 2 eq, MW: 114.5, d: 1.452) of methanesulfonyl chloride (MsCl) and 0.54 mL (6.87 mmol, 3 eq, MW: 101.2, d: 0.7255) of TEA and stirred for 30 min. The solution was then washed by water and saturated NaCl solution, respectively, and dried over anhydrous MgSO₄, filtered and evaporated. After purified by silica gel column, the product was obtained as white foam.
3’,5’-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-N3-(tert-Butoxycarbonyl)-2’SeMe-arabinothymine (6) In a vacuumed and argon purged round bottom flask, 0.084 mL (0.88 mmol, 6 eq, MW: 188, d: 1.987) dimethyl diselenide and 0.23 mL (0.59 mmol, 6 eq, C: 2.5 M in hexane) were added to 1 mL of THF under 0 ℃ and stirred for 30 min. It was then warmed up to room temperature and added THF dissolved 100 mg (0.15 mmol, MW: 678.94) of Compound (5) which was previously dried under vacuum for 20 min. After 2 hrs, there was no reaction. The solution was heated up to 45 ℃ and stirred overnight. The solution was diluted by DCM, washed by water and saturated NaCl solution, dried over MgSO₄, filtered, and evaporated. After silica gel column purification, ¹H NMR data showed that Boc was removed while SeMe failed to attach.

2.1.1 Failure Hypothesis

![Figure 2.2 Interaction Mechanism Between Lithium Ion and Boc Group](image)

Li⁺ could interact with carbonyl group on Boc, which facilitated its removal. The mechanism is shown above (Figure 2.2). See Chapter 2.2 for corresponding solution.
2.2 NaBH₄ as Reducing Agent

![Chemical structure](image)

Figure 2.3 SeMe Introduction Scheme Using NaBH₄

Sodium ion is more ‘gentle’ compared to Lithium ion. If it were Li⁺ that was removing Boc, NaBH₄ was supposed to solve the problem.

3',5'-O-((1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-N3-(tert-Butoxycarbonyl)-2'SeMe-arabinothymine (6) 22.3 mg (0.6 mmol, 4 eq, MW: 37.8) of sodium borohydride was placed in a round bottom flask and dried under vacuum for 20 min. It was then added 1 mL THF, 0.084 mL (0.88 mmol, 6 eq, MW: 188, d: 1.987) dimethyl diselenide, and 0.5 mL reagent alcohol and stirred for 30 min. To the solution 100 mg (0.15 mmol, MW: 678.94) of Compound (5) which was previously dried under vacuum for 20 min was added. After 2 hrs, there was no reaction. The solution was heated up to 45 °C and stirred overnight. The solution was diluted by DCM, washed by water and saturated NaCl solution, dried over MgSO₄, filtered, and evaporated. After silica gel column purification, ¹H NMR data showed that Boc was still removed while SeMe was introduced to α position.

2.2.1 Failure Hypothesis

First, steric hinderance was possible because sugar ring was too stiff due to 3',5' protection. Therefore, bulky base portion hinders SeMe to attack from β position as shown in
Figure 2.4. Therefore, 3',5' protection can be opened to test this hypothesis. See Chapter 2.3 and 2.4 for corresponding solutions.

Second, it was also possible that the removal of Boc would result in 2-O a good nucleophile which could attack 2'-C, and the self-cyclization left only α position for SeMe\textsuperscript{[7]}. It was observed that heating was needed for SeMe introduction in the initial attempt scheme, and it was suspected that heating would also facilitate the removal of Boc. Therefore, either the activation energy needed to be lowered to avoid heating, or the N3 protecting group needed to be stable enough not to be removed. See Chapter 2.5, 2.6, and 2.7 for corresponding solutions.
2.3 Protection on 5' Position\textsuperscript{[8]}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{scheme}
\caption{Scheme for 5' Trityl Protection}
\end{figure}

**2'-O-Mesityl-N3-(tert-Butoxycarbonyl)-thymine (7)** 500 mg (0.74 mmol, MW: 678.94) of Compound (5) was placed in a round bottom flask, dried under vacuum for 20 min, applied argon balloon, and dissolved in 7 mL of THF. It was added 0.18 mL (1.1 mmol, 1.5 eq, MW: 161.21, d: 0.989) triethylamine trihydrofluoride and stirred under room temperature. After complete reaction (determined by TLC), the solution was diluted by DCM, washed by water and saturated NaCl solution, dried over MgSO\textsubscript{4}, filtered, and evaporated. After silica gel column purification, the product was obtained as white foam.

**5'-O-Trityl-2'-O-Mesityl-N3-(tert-Butoxycarbonyl)-thymine (8)** 100 mg (0.23 mmol, MW: 436.43) of Compound (7) was placed in a round bottom flask, dried under vacuum for 20 min, applied argon balloon, and dissolved in 5 mL of pyridine. It was then added 93.5 mg (0.28 mmol, 1.2 eq, MW: 338.83) of 4,4'-dimethoxytrityl chloride (DMTrCl). After complete reaction (determined by TLC), the solution was diluted by DCM, washed by 3M HCl, saturated NaHCO\textsubscript{3} solution, and saturated NaCl solution, dried over MgSO\textsubscript{4}, filtered, and evaporated. After silica gel column purification, the product was obtained as white foam.

SeMe introduction to Compound (8) still failed with Boc removed while SeMe attacked from $\alpha$ position.
2.4 Protection on 3’ Position\textsuperscript{[9]}

![Scheme for 3' TIPDSOH Protection](image)

2’-O-Mesyl-3’-O-(1,1,3,3-Hydroxy-Tetraisopropyldisiloxane-1,3-diyl)-N3-(tert-Butoxycarbonyl)-thymine (9) 200 mg of Compound (5) was placed in a round bottom flask and dissolved in 4 mL of THF, and then added 2 mL aqueous TFA (TFA:H\textsubscript{2}O=1:1) at 0 ℃. After complete reaction under 0 ℃ (determined by TLC), the reaction solution was neutralized with saturated NaHCO\textsubscript{3} and diluted with EA. The organic layer was then washed with water and saturated NaCl solution, dried over MgSO\textsubscript{4}, filtered, and evaporated. After silica gel column, the product was obtained as white solid.

SeMe introduction to Compound (9) still failed with Boc removed while leaving group was still attached.

2.5 Triflate as Leaving Group

![Scheme for Triflate as Leaving Group](image)
2′-O-Triflyl-3′,5′-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diy1)-N3-(tert-Butoxycarbonyl)-thymine (10) 500 mg (0.83 mmol, MW: 600.86) of Compound (4) was placed in a round bottom flask, dried under vacuum for 20 min, and applied argon balloon. It was then dissolved in 25 mL THF and added 101 mg (0.83 mmol, MW: 122.2) of DMAP, 0.17 mL (1.25 mmol, 1.5 eq, MW: 101.19, d: 0.726) of TEA, 0.1 mL (1.0 mmol, MW: 168.5, d: 1.583) of trifluoromethanesulfonyl chloride (TfCl).

According to TLC, there were too many side products with significant percentage, and the solution was discarded because it was not efficient to purify and identify each product.

2.6 Trityl as Base Protecting Group

![Diagram](image)

Figure 2.9 Scheme for Trityl as Base Protecting Group

2′-O-Trimethylsilyl-3′,5′-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diy1)-N3-Trityl-thymine (11) 100 mg (0.17 mmol, MW: 572.9) of Compound (2) was placed in a round bottom flask, dried under vacuum for 20 min, and applied argon balloon. It was then dissolved in 4 mL of THF and added 88.7 mg (0.26 mmol, 1.5 eq, MW: 338.83) of DMTrCl, and 43.7 mg (0.26 mmol, 1.5 eq, MW: 166.91) of silver acetate or 4.2 mg (0.17 mmol, 1 eq, MW: 24) of sodium hydride.

According to TLC, there was no reaction. Trityl group might be too bulky to be introduced to base.
2.7 Benzyl as Base Protecting Group

![Chemical Structures]

Figure 2.10 Scheme for Benzyl as Base Protecting Group and SeMe Arabino Introduction

First, the initial steps were simplified, where TMS protection was no longer needed and Ms leaving group was introduced right after 3′,5′-TIPDS protection.

3′,5′-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-thymine (12) 5g (19.4 mmol, MW: 258.23) of 5-Methyluridine (1) was placed in a round bottom flask and dried under vacuum for 20 min, and applied argon balloon. It was then dissolved in 120 mL of pyridine and placed in ice bath. 4.4 mL of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (20.4 mmol, 1.05 eq, MW: 315.43, d: 1.454) was added dropwise while stirring. The flask was removed from ice bath and stirred for 3 hrs at room temperature. After reaction completed (determined by TLC), the solution was evaporated to remove most of pyridine and then diluted by dichloromethane (DCM), and was then washed by 3M HCl, saturated sodium bicarbonate (NaHCO₃) solution, and saturated NaCl solution, respectively. It was then dried with MgSO₄ and filtered and evaporated. After purified by silica gel column, the product was obtained as white foam.

2′-O-Mesyl-3′,5′-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-thymine (13) 1 g (2.0 mmol. MW: 500.74) of Compound (12) was dried under vacuum for 20 min and applied argon balloon. It was then dissolved in 20 mL DCM and added 0.32 mL (4.0 mmol, 2 eq, MW: 114.5, d: 1.452) of methanesulfonyl chloride (MsCl) and 0.84 mL (6.0 mmol, 3 eq, MW: 101.2, d: 0.7255) of TEA and stirred for 30 min. The solution was then washed by water and saturated
NaCl solution, respectively, and dried over anhydrous MgSO₄, filtered and evaporated. After purified by silica gel column, the product was obtained as white foam.

2’-O-Mesy1 -3’,5’-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-N3-Benzyl-thymine (14) 500 mg (0.86 mmol, MW: 578.82) of Compound (13) was placed in a round bottom flask and dried under vacuum. It was then dissolved in 5 mL dimethylformamide (DMF) and added 0.36 g (2.58 mmol, 3 eq, MW: 138.21) of potassium carbonate and stirred for 2 hrs. The solution was then added 0.15 g (0.86 mmol, 1 eq, MW: 171.03) of benzyl bromide (BnBr) and stirred overnight at room temperature. The mixture was then diluted with EA and washed with water and saturated NaCl solution, and the organic layer was dried over MgSO₄, filtered, and evaporated. After silica gel column, the product was obtained as white solid.

3’,5’-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-N3-Benzyl-2’-SeMe-arabinothymine (15) In a vacuumed and argon purged round bottom flask, 0.084 mL (0.88 mmol, 6 eq, MW: 188, d: 1.987) dimethyl diselenide and 0.23 mL (0.59 mmol, 6 eq, C: 2.5 M in hexane) were added to 1 mL of THF under 0 ℃ and stirred for 30 min. It was then warmed up to room temperature and added THF dissolved 100 mg (0.15 mmol, MW: 668.95) of Compound (5) which was previously dried under vacuum for 20 min. The solution was heated up to 55 ℃ and stirred overnight. The solution was diluted by DCM, washed by water and saturated NaCl solution, dried over MgSO₄, filtered, and evaporated. After silica gel column purification, the product was obtained as white solid. ¹H NMR and mass spectrometry data showed positive results for desired product.
3 RESULTS

3.1 $^1$H NMR Analysis

Figure 3.1 $^1$H NMR Spectrum for 3',5'-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-N3-Benzyl-2'-SeMe-arabinothymine
The sign for successful SeMe arabino introduction is 1’-H doublet. As shown above (Figure 3.2), 1’-H peak has changed from a singlet (red peak from Compound 14) to a doublet with J=7.2, indicating a strong coupling between 1’-H and 2’-H. If SeMe were at α position, 1’-H and 2’-H should have been about 90 degrees with J value almost 0.

*Figure 3.2 Enlarged $^1H$ NMR Graph Focusing on 1'H*

*Figure 3.3 1’-H and 2’-H Relative Position When SeMe Introduced from Different Directions*
3.2 Mass Spectrometry Analysis

Mass spectrum suggested the existence of desired product. Also, as shown below (Figure 3.5), selenium isotopic distribution was consistent with natural abundance and predictions from ChemDraw.

Figure 3.4 Mass Spectrum for 3’,5’-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-N3-Benzyl-2’-SeMe-arabinothymine.

HRMS (ESI): Calc. for C30H48N2O6SeSi2 [M+H]+:669.2294, found: 669.2497

Figure 3.5 Enlarged Mass Spectrum Focusing on Selenium Isotopic Distribution
4 CONCLUSION

The final scheme is now finished, with the first step simplified, without needing to use TMS protection. Through the discovery of the synthesis path, the mechanism of each step was analyzed. The key step is N3 protection where a stable protecting group is needed to prevent thymine’s self-cyclization due to N3 protecting group removal resulting in α position introduction of SeMe.

Future works include deprotection, synthesis of corresponding phosphoramidite and oligo sequences, crystallization and structural determination. Although this is just a small step in the big picture, it opens up a whole new area to explore. Selenium modified NAs will potentially be broadly utilized in diagnosis and therapeutics, targeting the source of diseases with the least dosage and highest precision level compared to existing protein targeting drugs.
REFERENCES


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APPENDICES

Appendix A. Mechanisms for Key Reactions

i. 3’,5’-TIPDS Protection:

![Diagram of 3’,5’-TIPDS Protection]

ii. 2’-OTMS Protection:

![Diagram of 2’-OTMS Protection]

iii. N3-Boc Protection:

![Diagram of N3-Boc Protection]
iv. 2’-OTMS Deprotection:

\[ \text{R1} \overset{\text{H}^+}{\rightarrow} \text{R2} \]

v. 2’-OMs Leaving Group Addition:

\[ \text{R1} + \text{OMs} \rightarrow \text{R2} \]

vi. 5’-DMTr Protection:

\[ \text{R1} \overset{\text{H}^+}{\rightarrow} \text{R2} \]
vii. N3-Bn Protection:

![Chemical structure](image1)

viii. 2'-SeMe Introduction:

![Chemical structure](image2)